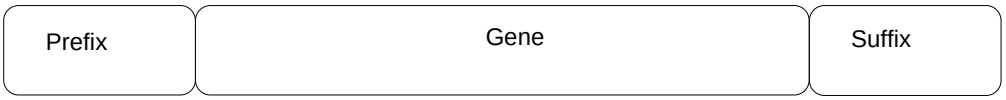
Protocol for small segments synthesis by PCR

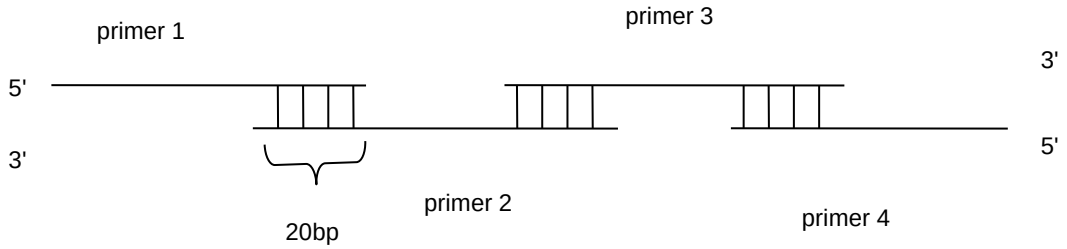
Description:

There are some parts we cannot find in the Kit Plates and whose length is too short to get them from purification. So, we designed a method to synthetize this kind of small segments: design 4 primers which have almost 20 base pairs overlapping sequence and conducted twice PCR, and finally put the segment into a vector by double digestion and ligation.

The whole gene:



4 designed primers:



First PCR:

Mixture: primer 1, primer 2, primer 3, primer 4, dNTP, 10×Buffer;

Pfu DNA polymerase;

Tm ： 35℃~50℃;

Circulation：22;

Gel Purification with AxyPrep DNA Gel Extraction Kit;

Second PCR ：

Template: product of first PCR;

Primer 1, primer 4;

Pfu DNA polymerase;

dNTP;

10×Buffer;

Tm : 60℃~65℃;

Circulation: 34;

Gel Purification with AxyPrep DNA Gel Extraction Kit;

Double enzyme digestion and ligation

Note:

Length of primers: 32~36bp

Length of overlapping sequence: 17~22bp